ANTIGENS OF THE SEA URCHIN SPERM SURFACE[1]

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Morphological and physiological evidence indicates that the initial steps in fertilization involve the sperm and the egg surfaces. Furthermore, these steps would appear to be chemical interactions between surface substances. Beginning with the pioneer studies of F. R. Lillie (1913, 1914), the chemical relationships have been visualized as between complex molecules; and antigen-antibody systems have served as useful models (see Tyler, 1948). Most of the present information has been obtained from studies on agents extracted from sperm and eggs. Notable among these agents are the sperm isoagglutinin, fertilizin, obtained from the egg jelly layer and the egg agglutinating antifertilizin obtained from sperm. Although these and other agents have been studied extensively, their role in fertilization has not been clearly defined (see Metz, 1957a, for review). On the other hand, relatively little effort has been directed toward analysis of the intact cell surface. Contributions in this direction include Tyler's (1946) study of the effect of specific antiserum on the fertilizing capacity of sea urchin sperm. In this study non-agglutinating, univalent antibody prepared against sperm antifertilizin was found to reduce the fertilizing capacity of sea urchin sperm. In an analogous investigation on *Paramecium,* antiserum was found to block initial steps in the mating process (Metz, 1954). Extension of this line of study would seem to promise significant information for an understanding of the interaction of egg and sperm surfaces at fertilization. As a step in such an investigation it seemed desirable to map out the antigenic structure of the *Arbacia* sperm surface. Accordingly, in the present study an effort was made to determine the number of sperm surface antigens and their distribution with respect to the morphology of the sperm, and to examine for sperm surface antigens that do not appear in sea water extracts.

Studies on the antigenic structure of sperm are found in publications extending over a period of 60 years. However, most of such studies were not directed primarily toward an understanding of the fertilization process. More recently Mudd and Mudd (1929), Henle (1938), Snell (1944), Smith (1949) and Pernot (1957) among others have demonstrated species, strain and tissue specificities. Certain of these studies, notably those of Henle *et al.* (1938) and Pernot (1957), are also concerned with the number and distribution of sperm antigens. The former study showed three agglutinogens on bull sperm, one confined to the tails, one restricted to heads and the third common to both. Pernot's (1957) agar diffusion and immunoelectrophoretic study of guinea pig material revealed eleven antigens in

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the seminal plasma and seven in alkaline sperm tail extracts. Six antigens were common to both materials. Pernot also presents some evidence for an antigen common to both heads and tails, and an experiment suggesting another antigen restricted to the guinea pig sperm tail. It remains to be shown which if any of the sperm antigens in Pernot's extracts are components of the cell surface. Finally, Dallam and Thomas (1953) have isolated lipoprotein from sperm heads of several mammalian species. Antisera prepared against such lipoprotein agglutinated sperm.

These studies concern mammalian material. Among echinoderms extensive serological investigations of the egg have been made by Tyler and Brookbank (1956a, 1956b), and the Perlmanns (Perlmann, 1957; Perlmann and Perlmann, 1957a, 1957b; see Runnström *et al.,* 1959, for review). Studies of the sperm include a brief reference by Perlmann (1957) and accounts by Tyler and O'Melveny (1941) and Tyler (1946, 1949). In the last report Tyler (1949) lists cross-agglutination reactions between antiserum and sperm of several species and demonstrates a minimum of two antigens on the *Strongylocentrotus purpuratus* sperm surface.

Material and Methods

Seven echinoderms were employed in the study. These include *Arbacia punctulata, Lytechinus variegatus, Mellita quinquiesperforata* and *Plagiobrissus grandis* from the vicinity of the Florida State University Marine Laboratory at Alligator Point, Florida, and *Arbacia punctulata, Echinarachnius parma* and *Asterias forbesii* from Woods Hole, Mass.

Gametes were obtained from *Arbacia* by electrical stimulation (see Harvey, 1956) or by excising the gonads. Treatment with isotonic KCl was avoided since this method causes release of the antigen-containing dermal secretion (Metz, 1959). Gametes were obtained from *Lytechinus, Mellita, Echinarachnius* and *Plagiobrissus* by treating the gonads with isotonic KCl.

Sperm and seminal plasma were separated by low speed centrifugation. The sperm was re-suspended once in sea water and centrifuged again. The supernatant was discarded and the settled sperm was made up as a 25% suspension by addition of three volumes of sea water. This stock suspension was used for injections and absorptions. For spot plate agglutination tests a 1% sperm suspension was prepared by diluting the stock suspensions to $\frac{1}{25}$ with sea water.

Injections. To obtain antisera five rabbits were injected with *Arbacia* sperm (25% washed sperm in sea water) or sperm extracts, three rabbits were injected with *Lytechinus,* one with *Echinarachnius* and three with *Asterias* sperm. The immunizing antigens were administered through intravenous, intraperitoneal and subscapular injections. In the last instance the antigen was injected as an emulsion in Freunds' adjuvant (Difco). This yielded the antisera with the highest sperm agglutinin titers. Most of the rabbits received two or three courses of injections.

Although anti-*Arbacia* sera from several rabbits were examined with uniform results only experiments with antiserum from the hyper-immune rabbit "#33" are recorded here. This rabbit was injected with antigen in Freunds' adjuvant. The animal was bled two weeks subsequent to the injection. The immune serum obtained regularly agglutinated sperm to dilutions of 2^{-8} to 2^{-10}. No sera were pooled.

Tests, assays and absorptions. The sperm agglutination tests were performed by mixing one volume (usually one drop) of 1% sperm suspension with two volumes (2 drops or 0.1 ml.) of serum in depression slides. The salt concentration of sera was adjusted to that of sea water by dialysis or addition of an equal volume of 1.73 × sea water. Reactions were recorded after the mixtures had remained for three hours at room temperature. Parallel tests with control (preinjection) serum were performed in all experiments to control for nonspecific reactions.

The agglutination of *Arbacia* sperm with antiserum gave a regular pattern. When diluted serially in sea water the antiserum gave titers with sharp, reproducible end points. However, the morphology of the agglutinates varies upon dilution of the antiserum. Antiserum at dilutions to 2^{-5} yielded net-like agglutinates involving head-to-head, head-to-tail and tail-to-tail agglutination. Agglutination at higher antiserum dilutions was head-to-head, the sperm was motile and the agglutinates were "bouquet-like" in structure. Sperm in such dilute antisera fertilized eggs and became more active on addition of fertilizin. This last effect was associated with a breakdown of the antiserum agglutinates.

The absorptions were done with varying amounts of material. Absorbing antigen and antibody were usually allowed to react for three hours at room temperature. Absorption over a longer time did not result in lower titers.

Isolation of sperm tails and heads. Isolated sperm tails and heads were used in absorption and agglutination experiments. In order to separate sperm tails and heads washed, living or formalin-killed sperm (25% suspension) was subjected to the shaking action of the Mickle disintegrator for 20 minutes at room temperature. The resulting material was then centrifuged at low speed at 4° C. to sediment the sperm heads. Suspensions of tails and of heads were washed when necessary by high and low speed centrifugation, respectively, at 4° C.

Results

In this investigation primary interest centers about the sperm "surface" as opposed to "subsurface" or interior material with antigenic properties. Although the sperm surface may be difficult to define or delimit at the molecular level, present requirements are satisfied by an operational definition. According to this definition, sperm surface antigens (specific combining sites) are those antigens so situated that their combination with antibody under appropriate conditions results in sperm agglutination. In any event information concerning the role of the sperm surface in fertilization is the ultimate objective here and antigenic groups that are available for participation in the agglutination reaction should be sufficiently accessible for interaction with the egg surface in the initial stages of fertilization.

In the experiments described below some attention is given to the antigenic composition of sperm extracts. It is clear that the "surface" vs. "subsurface" origin of soluble antigens in sperm extracts is not readily established. For example, a single antigenic group might be readily extracted from the cell "interior" and at the same time exist in bound, unextractable and insoluble form at the cell "surface." However, removal of sperm agglutinating antibodies from antisera by absorption with sperm extracts serves to identify and separate antibodies against sperm surface antigens irrespective of the origin of the absorbing antigens.

Such absorbed sera in turn can serve to separate and identify sperm surface antigens. The experiments described below should be considered in this conceptual framework.

A) *Identification of sperm surface antigens by interspecific reactions*

In order to identify sperm surface antigens, cross-absorption tests were performed in interspecific, agglutinating combinations of sperm and antisera. Six foreign sperms were tested for cross-reaction against antisera prepared against sperm of four of the species. With the exception of the combination *Mellita* sperm

TABLE I

Sperm agglutinating action of antisera before and after absorption with sperm of various species

Sera		Immune sera Tested with					
Prepared against sperm	Abs. with sperm	A*	L	E	M	P	Asterias
A*	—	+	+	+	0	+	0
A	A	0	0				
A	L	+	0	0			
A	E	+	+	0			
A	E + L	+	0				
L	—	+	+	+	+(weak)	+	+(weak)
L	L	0	0				
L	A	0	+	0			
L	E	+	+	0			
L	E + A	0	+	0			
E	—	+	+	+	+		+
E	L	0	0	+			
E	A	0	+				
E	A + L	0	0	?			
Asterias	—	0	0		0		+

*A = *Arbacia;* E = *Echinarachnius;* L = *Lytechinus;* M = *Mellita;* P = *Plagiobrissus.*
Control (pre-injection) sera failed to agglutinate sperm in parallel tests to those in the table.

vs. *Arbacia* antiserum, cross-reactions were found among all echinoid combinations tested. These relations are summarized in Table I.

Certain of these cross-reacting combinations were used in cross-absorption experiments. These included the series *Arbacia, Lytechinus* and *Echinarachnius* as seen in Table I. Absorption with the homologous sperm rendered antisera non-agglutinating for the cross-reacting heterologous species. In every case complete absorption with an heterologous sperm yielded a serum which still agglutinated the homologous species. Furthermore, in most cases such absorbed sera agglutinated the other heterologous species as well. Thus anti-*Arbacia* serum absorbed with *Echinarachnius* sperm still agglutinated *Lytechinus* and *Arbacia* sperm.

Evidently, then, *Lytechinus* and *Arbacia* sperm possess surface antigens lacking on *Echinarachnius*. A further absorption of the *Echinarachnius* sperm absorbed anti-*Arbacia* serum with *Lytechinus* sperm yielded a serum which failed to agglutinate *Lytechinus* but still agglutinated *Arbacia* sperm. Accordingly, it is concluded that the Arbacia sperm surface possesses at least three antigens or antigenic complexes, one specific for *Arbacia,* a second shared with *Lytechinus* and a third shared with *Echinarachnius*. It is of some interest that *Lytechinus* sperm absorbs all *Echinarachnius* sperm agglutinins from anti-*Arbacia* sperm serum. This indicates that all the "*Arbacia* antigens" present on the *Echinarachnius* sperm surface are also present on the *Lytechinus* sperm. Comparable results were obtained in absorption experiments using anti-*Lytechinus* sperm serum (Table I).

B) *Sperm surface antigens in sperm extracts*

In the previous section the *Arbacia* sperm surface was shown to possess at least three antigens or antigenic complexes. In view of the possible role of insoluble as well as soluble sperm surface substances in fertilization (see Metz, 1957a), it seemed of interest to examine sperm extracts for soluble antigens in common with sperm surface antigens. This matter is of special interest because it concerns the question whether the antifertilizin extracts from sperm contain the full complement of sperm surface antigens. In one series of experiments soluble antigen extracts were prepared by freeze-thawing *Arbacia* sperm. This is one method for preparing sperm antifertilizin (Tyler, 1939). All extracts were subject to low speed centrifugation (3000 × g for 20 minutes) to remove fragments of the sperm surface. Certain preparations were also subjected to high speed centrifugation (26,000 × g) to remove the larger sub-cellular particulates. Constant amounts of anti-*Arbacia* sperm serum were then absorbed with increasing amounts of the sperm extracts. The absorbed sera were then assayed for sperm agglutinating action. As seen in Figures 1a, 3a, 4a, and 6a, increasing amounts of sperm extract lowered the sperm agglutinin titer until a constant value was reached. Beyond this point increasing amounts of extract fail to affect the sperm agglutinating action. The extracts failed to remove all of the sperm agglutinins (seven experiments). Accordingly, it appears that the extracts do not contain all of the sperm surface antigens. Some of these evidently are not extractible in sea water by freeze-thawing and do not appear as components in extracted "antifertilizin." Most of these antigens are heat-labile. If the frozen-thawed extract is heated a precipitate begins to form at 55° C. and after further heating for one minute at 100° C. the supernatant has reduced antifertilizin activity and lowers the sperm agglutinating titer of antiserum only one dilution step (compare Figure 1, a and b).

In view of the antibody absorbing action of extracts prepared by freeze-thawing sperm, it seemed of interest to examine extracts prepared by other methods for antigens in common with those present on the intact sperm surface. Accordingly, extracts were prepared by heating (100° C. for one minute) followed by centrifugation, by disruption in the Mickle disintegrator, and by acid extraction.

As seen in Figure 2a (typical of two experiments), extracts prepared by heating had little if any effect on the sperm agglutinating action of anti-sperm serum. Evidently, then, heat extraction yields few if any sperm surface antigens. However, antigenic material is present in extracts prepared by heating, for such extracts

produce one precipitin band in agar when diffused against antiserum (to be published). Possibly this is sub-surface material. If so, it suggests that the antifertilizin (egg jelly precipitating and fertilizin neutralizing) activity of extracts prepared by heating (Frank, 1939) is not due to sperm surface material. Finally, as seen above, frozen-thawed extracts contain insignificant amounts of surface antigenic material after heating to 100° C. Extracts prepared by action of the Mickle disintegrator also neutralized the sperm agglutinating action of anti-whole sperm serum. As seen in Figure 2c (two experiments), such absorption reduced the agglutinin titer to a constant level, but failed to remove all agglutinins.

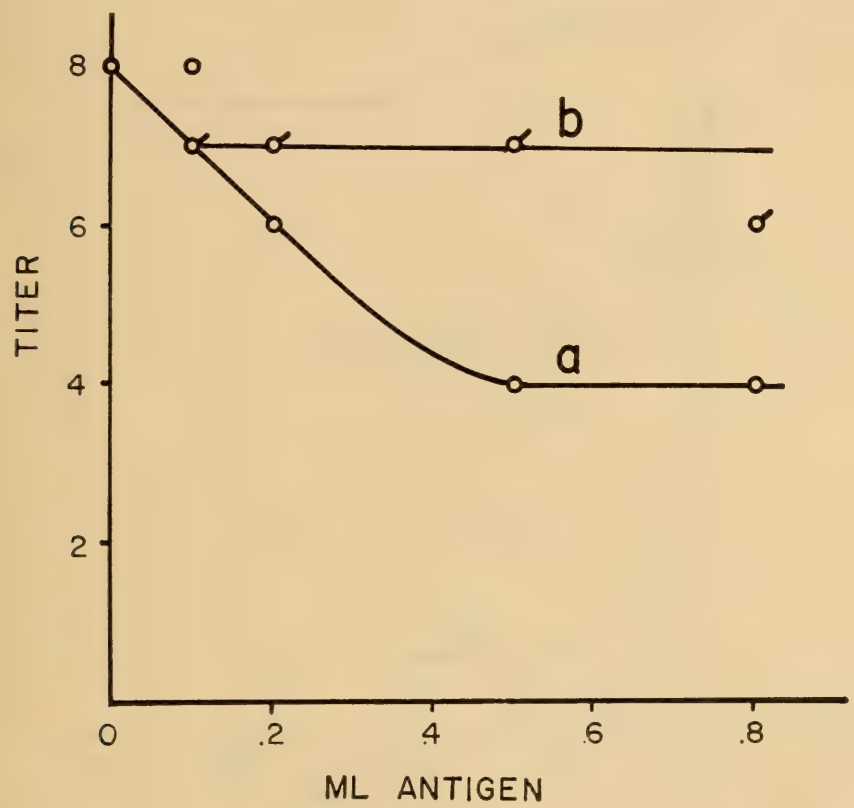

FIGURE 1. Effect of absorption with increasing amounts of antigen on sperm agglutinating action of anti-*Arbacia* sperm serum. Curve (a): Antiserum absorbed with frozen-thawed sperm extract, tested on *Arbacia* sperm. Curve (b): Antiserum absorbed with heated (100° C., one minute) frozen-thawed sperm extract, tested on *Arbacia* sperm. Ordinate: Titer $= -\log_2$ of highest antiserum dilution giving sperm agglutination. Abscissa: ml. antigen added to 0.5 ml. antiserum.

Acid extraction of sperm also yields antifertilizin (Tyler and O'Melveny, 1941). In one experiment anti-whole sperm serum was absorbed with increasing amounts of neutralized pH 3.0 sperm extract. Figure 2b again shows reduction of the agglutination titer to a constant level. In an additional comparative experiment sperm suspensions (12.5%) were extracted at pH 3.2, 7.2 and 9.0 for six hours. The extracts were subsequently tested for antibody absorbing action. The pH 7.2 ("aged sperm") extract lowered the sperm agglutination titer a maximum of one-half (one-fold) whereas the acid and alkaline supernatants lowered the titer of the antiserum a maximum of $\frac{1}{8}$ (three-fold). Thus, acid and alkaline extracts

of sperm, like frozen-thawed and Mickle extracts, contain some but not all of the antigenic groups present on the sperm surface.

Relation of antigens in sperm extracts to fertilizin. In view of the fact that the soluble antigen preparations contain antifertilizin, it seemed of interest to examine the relations of such extracts and of anti-sperm serum to fertilizin. Specifically, it appeared of interest to test for action of fertilizin on the antiserum neutralizing action of soluble sperm antigen extracts (antifertilizin). To test for neutralizing

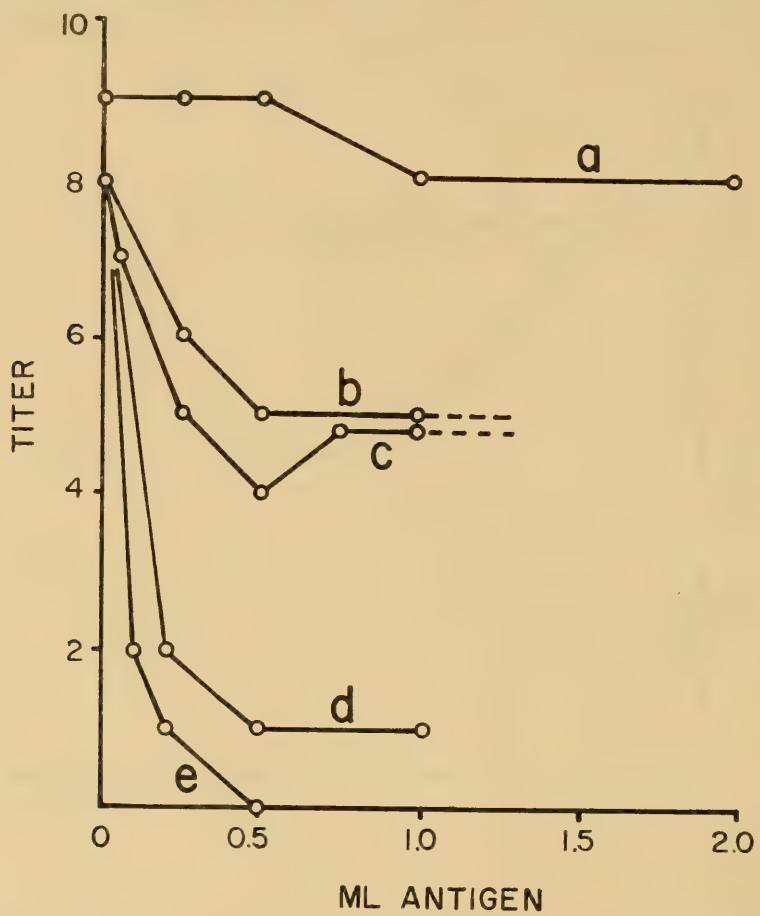

FIGURE 2. Effect of absorption with increasing amounts of antigen on sperm agglutinating action of anti-*Arbacia* sperm serum. Curve (a): Absorbing antigen prepared by heating (100° C., one minute) sperm. Curve (b): Absorbing antigen prepared by extracting sperm in pH 3.0 sea water. Curve (c): Absorbing antigen prepared by Mickle disintegration of sperm. Curve (d): Absorbing antigen: isolated sperm heads. Curve (e): Absorbing antigen: whole intact sperm. Absorbing antigen for curves (a), (b) and (c) centrifuged 3000 × g. Each curve represents a different experiment. Ordinate and abscissa as in Figure 1.

action, fertilizin and antiserum were mixed in varying proportions and the sperm agglutinin titers determined. The experiment is complicated by the fact that fertilizin and antiserum both agglutinate sperm. To avoid confusion from the action of the two different agglutinins, the sperm was examined for agglutination after sufficient time (three hours) to permit reversal of any agglutinating action of fertilizin (see methods section). As seen in Figure 3b the sperm agglutinin titer of the antiserum was not reduced by fertilizin. It appears, then, that the

sperm surface and fertilizin do not have antigenic combining sites in common which are essential for sperm agglutination.

On the other hand, the combining sites of the soluble antigens in frozen-thawed extracts evidently are blocked by fertilizin. This is again seen in Figure 3, curve c. In this experiment decreasing amounts of fertilizin were mixed with increasing amounts of the sperm surface extract (antifertilizin). The mixtures were subsequently used to absorb the anti-sperm serum. When the extract-to-fertilizin ratio equalled 0.143 (0.25 ml. extract) the mixture failed to affect the

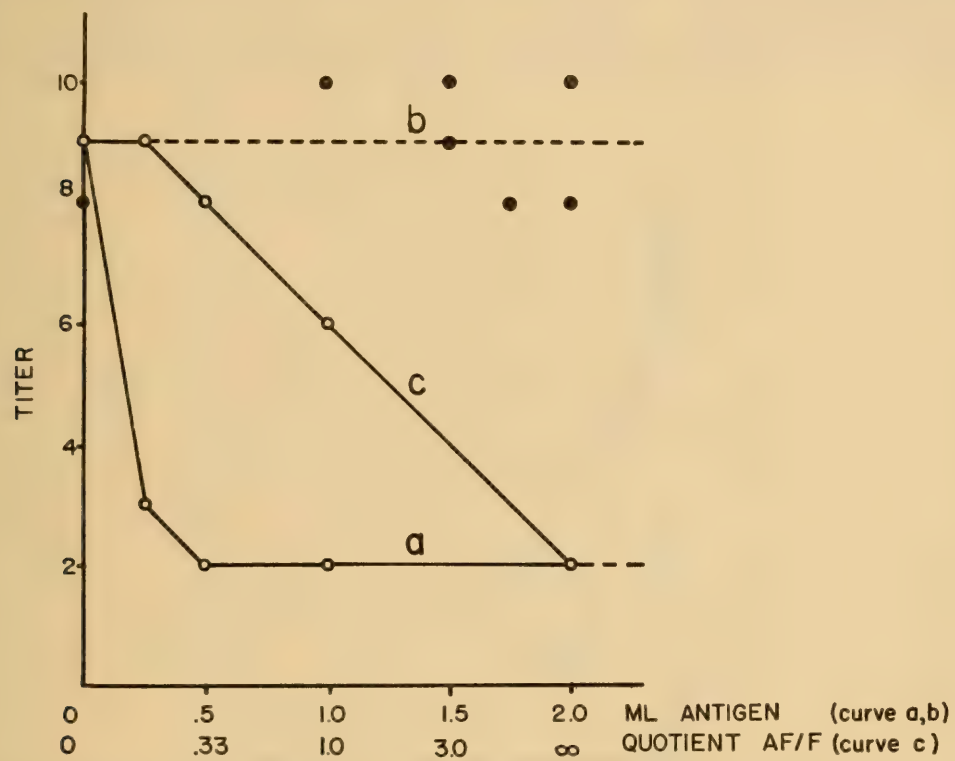

FIGURE 3. Effect of absorption with increasing amounts of antigen on the sperm agglutinating action of anti-*Arbacia* sperm serum. Curve (a): Serum absorbed with sperm extract (antifertilizin) prepared by freeze-thawing sperm and centrifuging at 3000 × g for 20 minutes. Curve (b): Antiserum absorbed with fertilizin. Curve (c): Antiserum absorbed with antifertilizin-fertilizin mixtures. Ordinate: Titer = $-\log_2$ of highest antiserum dilution giving sperm agglutination. Abscissa for (a) and (b): ml. antigen added to 0.5 ml. antiserum. For (c): Quotient antifertilizin/fertilizin/0.5 ml. antiserum. Two ml. fertilizin and no antifertilizin at quotient = 0. The same fertilizin preparation was used for curve b and c. Absolute amounts of antifertilizin, antisera and total volumes in curves (a) and (c) are identical along the abscissa.

sperm agglutinin titer of the anti-sperm serum, whereas the same amount of antifertilizin alone reduced the agglutinin titer six-fold. Increase in the proportion of antifertilizin resulted in mixtures with antiserum neutralizing action. This result shows that fertilizin neutralizes the ability of the frozen-thawed extract to absorb sperm agglutinins from the anti-sperm sera.

Distribution of "soluble" surface antigens. Data in the previous section show that extraction of sperm yields solutions of soluble sperm antigens and that these neutralize some of the antibodies (agglutinins) for the sperm surface. On the

other hand the extracts fail to neutralize all of the sperm agglutinating antibodies. Accordingly, the extraction procedures fail to remove some "insoluble" antigenic material from the sperm. Although the antigens in the preparation may not be extracted from the sperm surface they are antigenically related to, and therefore serve to identify, surface antigens. In an attempt to localize these "soluble antigens" on the sperm surface, antisera were treated with extracts prepared by freeze-thawing (antifertilizin) and then tested for agglutinating action on suspensions of isolated sperm heads, tails and intact sperm. As seen in Figure 4 such extracts completely neutralized the agglutinins for sperm tails. Evidently, then, the sperm tail surface possesses only soluble antigen. In this connection it is of interest that agar diffusion precipitin tests show only a single band when frozen-thawed extract of isolated

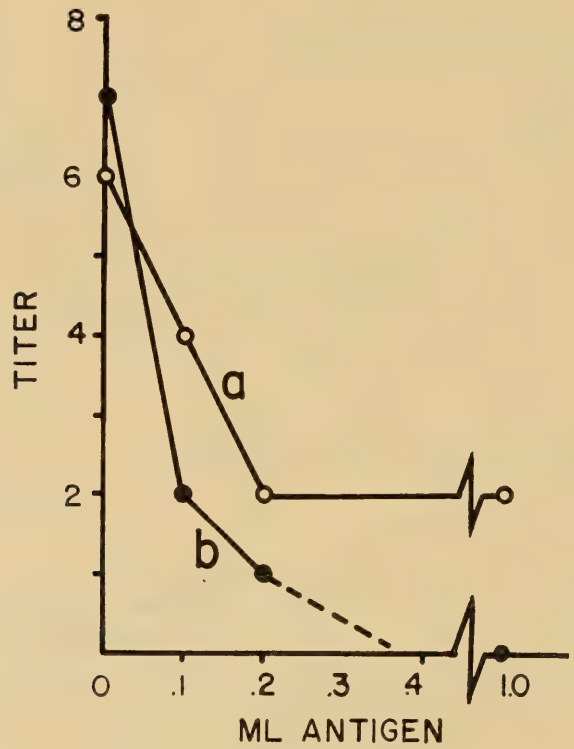

FIGURE 4. Agglutination of whole sperm and isolated sperm tails with anti-*Arbacia* whole sperm serum absorbed with frozen-thawed whole sperm extract (antifertilizin). Curve (a): Agglutination of whole sperm. Curve (b): Agglutination of isolated tails. Ordinate and abscissa as in Figure 1.

sperm tails is diffused against anti-whole sperm serum. This single band merges with one of four produced by whole sperm extract (to be published). These observations show that the insoluble antigens are confined to the sperm head. This view is confirmed by examination for agglutination of whole sperm and heads by the absorbed sera. As seen previously (Fig. 3a) the agglutinin titer for whole sperm is reduced to a constant level by absorption with frozen-thawed extracts. On the other hand, such extracts had no apparent effect on the ability of anti-sperm serum to agglutinate isolated sperm heads. Evidently, the surfaces of the isolated sperm heads and the frozen-thawed extracts do not have antigens in common and the sperm heads, prepared by treatment in the Mickle disintegrator, lack (have lost?) soluble surface antigenic material.

Regional localization of sperm surface antigens. In a previous section it was shown that whole sperm extract absorbed the agglutinins for isolated *Arbacia* sperm tails but failed to absorb all agglutinins for isolated sperm heads. This result indicates that the sperm tail antigens are soluble, whereas some or all of the antigens on the sperm head surface are insoluble. It seemed of interest to extend this study to a more direct analysis of differences between sperm heads and tails. To achieve this the method used by Henle *et al.* (1938) for bull sperm was employed. *Arbacia* sperm heads and tails were isolated by treatment in the Mickle disintegrator fol-

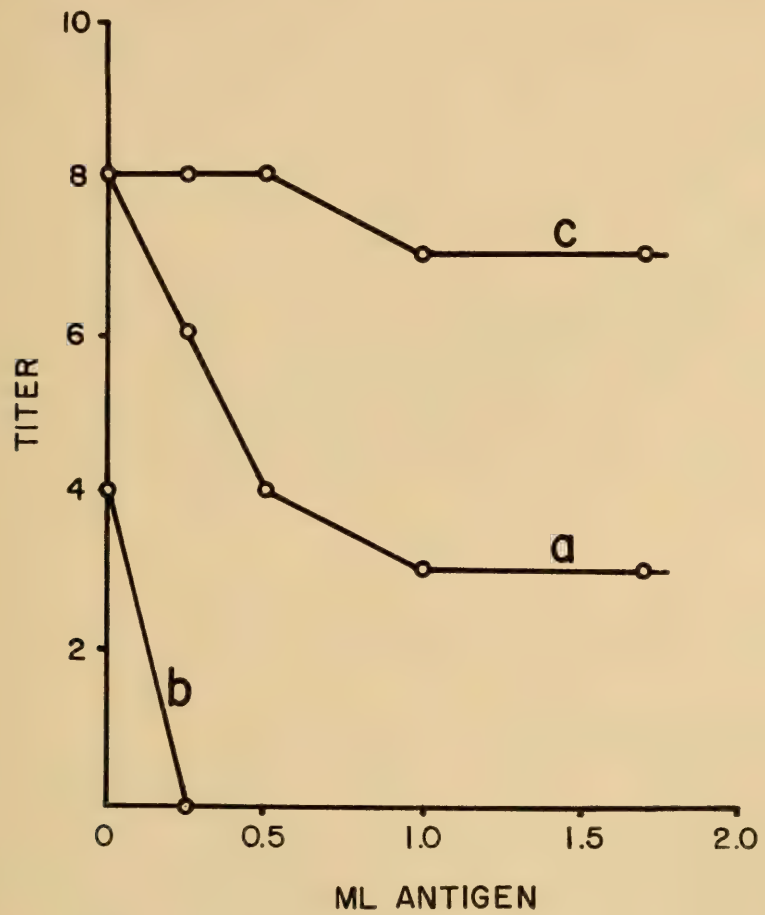

FIGURE 5. Sperm agglutinating action of anti-*Arbacia* whole sperm serum after absorption with sperm tails isolated from formalin-killed sperm. Curve (a): Tested on living *Arbacia* sperm. Curve (b): Tested on sperm tails isolated from formalin-killed sperm. Curve (c): Tested on sperm heads isolated from formalin-killed sperm. Ordinate and abscissa as in Figure 1.

lowed by differential centrifugation. The suspensions of isolated heads and tails were then used to absorb anti-whole sperm serum. Preliminary experiments showed that isolated tails always reduce the sperm agglutination titer in absorption experiments. Isolated sperm heads, however, showed varying results and require further study. The inconsistencies may be related to the presence of very readily soluble sperm surface antigens which appear in washings of the sperm.

Unfortunately, treatment of living sperm in the Mickle disintegrator resulted in considerable fragmentation of the sperm tails. This was especially true in the

preparation of large amounts of tails for absorption experiments. To facilitate isolation of unbroken tails sperm were first fixed in 1% formalin, washed in saline and the tails shaken from the remainder of the sperm in the Mickle disintegrator.

In using formalin-fixed sperm it is assumed that the formalin treatment does not alter the antigenic structure of the sperm surface. This assumption is valid to the extent that both living and formalin-killed whole sperm and sperm fragments agglutinated to the same titer with anti-sperm serum and that living and formalin-fixed material showed parallel absorbing action in preliminary experiments. For

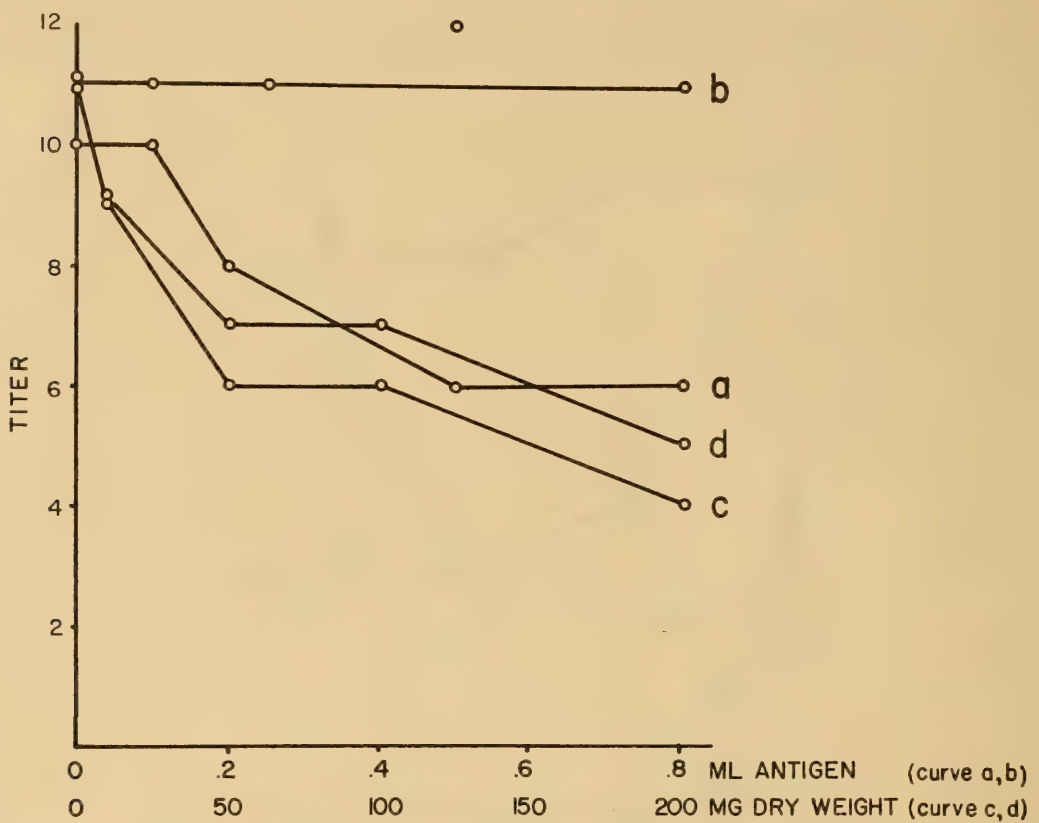

FIGURE 6. Sperm agglutinating action of anti-*Arbacia* whole sperm serum absorbed with *Arbacia* antifertilizin and sperm "ghosts." Curve (a) and (b): Absorbing antigen, frozen-thawed sperm extract. (a): Tested on *Arbacia* sperm. (b): Tested on *Lytechinus* sperm. Abscissa: ml. antigen added to 0.2 ml. antiserum. Curve (c) and (d): Absorbing antigen, frozen-dried "ghosts." (c): Tested on *Arbacia* sperm. (d): Tested on *Lytechinus* sperm. Abscissa: mg. dry weight. Ordinates for (a), (b), (c) and (d): Titer $= -\log_2$ of highest antiserum dilution giving sperm agglutination.

a final experiment washed sperm was fixed in formalin (1%), washed and treated for 30 minutes in the Mickle disintegrator. The tails were then isolated from the intact sperm and heads by centrifugation. Finally, the isolated tails were used in varying amounts to absorb the anti-whole sperm serum. This absorbed serum failed to agglutinate tails (Fig. 5b). However, it did agglutinate isolated sperm heads (Fig. 5c) and living whole sperm (Fig. 5a), but in both instances the absorbed serum showed lower titers than the unabsorbed serum. Evidently, then, the sperm heads possess a head specific antigen or antigens which are not present on sperm tails. The reduction in titer of sperm head agglutination by absorption

with tails indicates that some tail antigenic material is also present of the sperm heads.

Analysis of insoluble sperm surface antigens. In order to confirm the presence of water-insoluble sperm surface antigens it seemed of interest to attempt to absorb sera with sperm from which the soluble sperm antigens had been removed. Sperm "ghosts" were prepared by freezing 25% sperm, removing the supernatant, re-freezing the re-suspended residue and finally washing the insoluble material five times in sea water. As seen in Figure 6a, the initial extract of soluble antigens (antifertilizin) lowered the sperm agglutinin titer four-fold whereas the extracted "ghosts" when used in excess lowered the titer seven-fold (Fig. 6c). Evidently, then, the extracted "ghosts" possess insoluble antigens that are not present in the soluble fraction.

Furthermore, it is of interest that these *Arbacia* "ghosts" also lowered the sperm agglutinin titer for *Lytechinus* sperm (curve 6d), whereas the absorption with frozen-thawed extract (Fig. 6b) did not affect the titer for *Lytechinus*. It remains for future experiments to determine if all the antigens common to the two species are insoluble, and if the ones in the extracts are the specific antigens.

Presence of sperm surface components in body fluid and egg extract. Results from several experiments show that neither body fluid from male or female sea urchins nor egg extract contains sperm surface components involved in antiserum agglutination. Absorption of whole sperm antiserum with these extracts did not lower, or lowered only one step, the titer for sperm agglutination.

Discussion

In view of the striking morphology of the spermatozoan, the multiple antigen structure of cell surface generally (*e.g.* erythrocytes) and the presence of at least three distinct surface antigens on bull sperm (Henle *et al.,* 1938), and two on *Strongylocentrotus* sperm (Tyler, 1949), it is not surprising to find several antigens on the *Arbacia* sperm surface. The distinct head and tail antigens found on *Arbacia* sperm conform to the pattern found for bull sperm. It seems likely that tests of a wider range of heterologous species would reveal more cross-agglutination reactions between foreign sperm (*e.g.* Tyler, 1949) and the anti-*Arbacia* sperm serum. Absorption of the antiserum with such foreign sperm might reveal more antigens in addition to the three so far demonstrated on the *Arbacia* sperm surface. Furthermore, a more detailed analysis with such absorbed sera, such as testing for agglutinating action on isolated sperm heads and tails, should localize the individual antigens or antigenic complexes.

The number and distribution of the sperm surface antigens is not without interest, for this information contributes to the knowledge of sperm surface architecture. Furthermore, such antigens could be used as additional points of attack in studies on the role of the sperm surface in fertilization. However, the relationship of the sperm surface antigens to antigens present in sperm extracts appears to be of more immediate interest.

Extracts of sea urchin sperm prepared by heating (Frank, 1939), freeze-thawing (Tyler, 1939) and acid extraction (Tyler and O'Melveny, 1941) contain an agent or agents called antifertilizin, which neutralizes the sperm agglutinating action of fertilizin, agglutinates eggs and precipitates the egg jelly. This antifertilizin in

sperm extracts is assumed to come from the sperm surface and to include the specific sperm surface combining sites with which fertilizin reacts when it reversibly agglutinates sperm.

In support of this view Tyler (1949) extracted antifertilizin from the *Strongylocentrotus* sperm by acid treatment. Subsequent examination of the cells with the electron microscope revealed a partial breakdown of the sperm head surface, suggesting this as the site of origin of the antifertilizin. However, the situation is probably more complicated than this, for as seen in the present study absorption of anti-whole sperm serum with sperm extracts (antifertilizin) removes tail agglutinins but has little effect on the head agglutinins. This suggests that the antifertilizin may be present in large amounts on the sperm tail.

Furthermore, agar gel precipitin tests show that the antifertilizin extracts prepared by freeze-thawing regularly contain three to four antigens (to be published). The surface vs. sub-surface origin of these antigens is not immediately clear. However, the fact that this mixture of antigens will remove some sperm agglutinins from anti-sperm serum suggests that at least one of the antigens is of sperm surface origin. But even this experiment does not exclude the possibility that the absorbing antigen(s) possesses a combining group in common with the sperm surface but is actually extracted from the interior of the sperm.

In further attempts to identify these antigens the antigen neutralizing action of fertilizin was examined. Following treatment with fertilizin, antifertilizin failed to lower the sperm agglutination titer of the antiserum. Clearly, then, fertilizin can neutralize all the sperm surface antigens in the frozen-thawed extract. The fertilizin may be highly specific in action and neutralize only one of the four antigens. If this is the case, it follows that the extract prepared by freeze-thawing contains only one antigen in common with the sperm surface. In any event, the antigen or antigens involved appear to be related mainly to the antigens of the sperm tail (see above).

Studies on the role of specific egg and sperm substances in metazoan fertilization have been concerned mainly with soluble agents. It is of some interest, then, to find that extracts of sperm do not contain all of the surface antigens. Evidently, some antigens are either destroyed by the extraction procedure or remain in the insoluble residue. The second alternative seems the more likely in view of the nature of the extraction procedure. Indeed the absorption experiments with sperm "ghosts" show that insoluble antigens survive the extraction procedure. The role in fertilization of such insoluble sperm surface antigens remains to be investigated. The specific combining groups on the sperm surface that function in the antiserum agglutination reaction may not be identical with molecular configurations that function in fertilization. Nevertheless it seems likely that such material may form a part of, or be spatially related to, molecular surface patterns that do function in the union of sperm and egg. This apparently is the case in *Paramecium* (Metz, 1954).

Consideration here has been given only to the sperm head and tail. A more detailed study should take into account the subdivisions of these structures, such as the axial tip of the tail, and the midpiece and acrosome of the head. Furthermore, especial attention should be given to comparison of the antigens of the sperm before and after acrosomal filament discharge, for at least in some forms (*e.g.* starfish) activation of the egg results from interaction of the egg surface with the tip

of the acrosomal filament (see Metz, 1957b; Colwin and Colwin, 1957; Collier, 1959; Dan, 1956).

Summary

1. Sperm surface antigens are defined as those antigens which are detected by sperm agglutinating action of antisera.

2. Interspecific sperm agglutination tests show one or more sperm surface antigens common to all of the five echinoids tested except for a single combination.

3. The *Arbacia* sperm surface possesses a minimum of three antigens or antigenic complexes in common with other species. One of these is shared with *Lytechinus* sperm, a second with both *Lytechinus* and *Echinarachnius* and a third is not present on sperm of these species.

4. *Arbacia* sperm extracts prepared by freeze-thawing, Mickle disintegration or pH 3 treatment all contain "soluble" antigens in common with the sperm surface. The *Arbacia* sperm surface also possesses "insoluble" antigens which do not appear in the above extracts.

5. The "soluble sperm surface" antigen(s) in extracts is neutralized by fertilizin from *Arbacia* eggs.

6. It is present on the sperm tail surface and possibly to some extent on the sperm head.

7. The insoluble sperm surface antigen(s) is confined to the sperm head.

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